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CLAIMS

[Claim(s)]
[Claim 1] In the measuring method of the antigen-antibody reaction matter which measures the predetermined antigen in blood, or the concentration of an antibody, without separating a corpuscle from the extracted blood The solution which distributed the insoluble support particle to which sensitization of the antibody or antigen predetermined [aforementioned] to an antigen or an antibody was carried out, The xtracted blood and the solution which a surfactant is mixed at least and changes are mixed in simultaneous or arbitrary order. The measuring method of the antigen-antibody reaction matter characterized by irradiating light and carrying out the fixed quantity of the aforementioned predetermined antigen in the aforementioned blood, or the concentration of an antibody to the liquid of this reaction mixture based on the amount of transmitted lights.
[Claim 2] A predetermined antigen is the measuring method of the antigen-antibody reaction matter according to claim 1 characterized by being C reactivity protein.
[Claim 3] The wavelength of the light which irradiates the liquid of reaction mixture is 600 - 2000 nm. Measuring method of the antigen-antibody reaction matter according to claim 1 characterized by being in the range.
[Claim 4] The surfactant in a hemolysis diluted solution is the measuring method of the antigen-antibody reaction matter according to claim 1 characterized by being a nonionic surface active agent.
[Claim 5] Nonionic surface active agent, [Formula 1]
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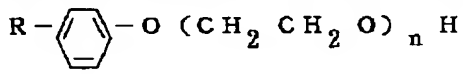

$$R = C_m H_{2m+1} \quad (1 \leq m \leq 18)$$ 、

Th measuring method of the antigen-antibody reaction matter according to claim 4 which comes out and is characterized by a certain thing.
[Claim 6] A nonionic surface active agent is polyoxyethylene-alkyl-ether R-O-(CH2 CH2 O) n HR=Cm H2m+1. (1<=m<=18) Measuring method of the antigen-antibody reaction matter according to claim 4 which comes out and is characterized by a certain thing.
[Claim 7] The measuring device of the antigen-antibody reaction matter which measures the predetermined antigen in blood, or the concentration of an antibody, without separating a corpuscle from th extracted blood which is characterized by providing the following Reaction cell A pouring means to pour in the solution which distributed the insoluble support particle by which sensitization of the antibody or antigen predetermined [aforementioned] to an antigen or an antibody was carried out to this reaction cell, the extracted blood, and the solution which blood and a surfactant are mixed at least among the solutions which a surfactant is mixed at least and change, and changes A mixed means to mix the liquid in the aforementioned r action cell An optical irradiation means to irradiate light at the liquid in the aforementioned reaction cell, an amount det ction means of transmitted lights to detect the amount of transmitted lights which penetrated the liquid among the light by this optical irradiation means, and a concentration fixed quantity means to ask for the aforementioned predetermined antigen in the aforementioned blood, or the concentration of an antibody based on the amount of transmitted lights which this amount d t ction means of transmitted lights detected

[Claim 8] A predetermined antig n is th measuring device of the antigen-antibody reaction matter according to claim 7 characterized by being C reactivity prot in.
[Claim 9] The wav length of the light which irradiates the liquid of reaction mixture is 600 - 2000 nm. Measuring d vice of the antigen-antibody reaction matter according to claim 7 characterized by being in th range.
[Claim 10] The surfactant in a h molysis diluent is the measuring device of the antigen-antibody reaction matter according to claim 7 characterized by being a nonionic surface active agent.
[Claim 11] Nonionic surface active agent, [Formula 2]
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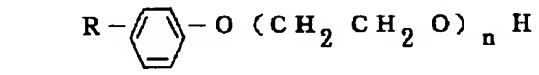

$R=C_mH_{2m+1}$ $(1 \leq m \leq 18)$ 、

The measuring device of the antigen-antibody reaction matter according to claim 10 which comes out and is characterized by a certain thing.
[Claim 12] A nonionic surface active agent is polyoxyethylene-alkyl-ether R-O-(CH2 CH2 O) n HR=Cm H2m+1. (1<=m<=18) Measuring device of the antigen-antibody reaction matter according to claim 10 which comes out and is characterized by a certain thing.

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DETAILED DESCRIPTION

[Detailed Description of the Invention]
[0001]
[The technical field to which invention belongs] this invention relates to the method of measuring the matter which produces antigen-antibody reactions, such as C reactivity protein [in blood] (it is called below C-Reactive Protein; CRP), rheumatoid factor (RF), and antistreptolysin O (ASO), and the equipment to measure.
[0002]
[Description of the Prior Art] There is latex condensation immunonephelometry using the insoluble support particle as a measuring method of this kind of matter. The detail about this method is indicated by each official report of JP,53-24015,A, JP,53-62826,A, JP,60-259964,A, and JP,60-259965,A. According to this lat x condensation immunonephelometry, the concentration of the matter which produces an antigen-antibody reaction can be quantitatively measured with a sufficient precision by very high sensitivity.
[0003]
[Problem(s) to be Solved by the Invention] However, by this method, if the extracted blood (whole blood) is used as it is, it cannot be influenced of an erythrocyte, hemoglobin, etc. and exact fixed quantity measurement cannot be performed. For this reason, in the conventional biochemical inspection, centrifugal separation of the blood extracted as mentioned above was carried out, the blood serum was taken, and it was measuring using this blood serum. For this reason, the inspection pin center,large is entrusted, and the long time is taken to know an inspection result also in case of emergency in the institution without a centrifugal separation machine. On the other hand, in order to have investigated the constituent of blood (the number of erythrocytes, and white blood cell count) of the extracted blood, it had taken time and effort very much that it must separate into the object for biochemical inspection and constituent-of-blood ch cking, and the blood which had to inspect using the plasma which added the anticoagulant into blood, therefore was extracted must be processed etc.
[0004] Then, the method of measuring without separating a corpuscle from blood is offered by JP,62-62291,B. However, according to this method, it is necessary to make the number of coexistence erythrocytes in reaction time very fewer than the particle number of the latex to be used. For this reason, since the object which became thin is measured, a problem is in the accuracy of measurement.
[0005] this invention was made in view of such a conventional fault, and the purpose is measuring the concentration of the matter which produces the antigen-antibody reaction in the blood using the same sample as using for constituent-of-blood inspection with a sufficient precision, without carrying out centrifugal separation of the extracted blood.
[0006]
[Means for Solving the Problem] In the measuring method of the antigen-antibody reaction matter which measures the predetermined antigen in blood, or the concentration of an antibody, without the measuring method of this invention separating a corpuscle from the extracted blood The solution which distributed th insoluble support particle to which sensitization of the antibody or antigen predetermined [aforementioned] to an antigen or an antibody was carried out, The extracted blood and the solution which a surfactant is mix d at least and changes are mixed in simultaneous or arbitrary order, light is irradiated at the liquid of this reaction mixture, and it is characterized by carrying out th fixed quantity of the aforementioned predetermined antigen in the aforementioned blood, or the concentration of an

antibody based on the amount of transmitted lights.
[0007] Wh n the insoluble support particle and blood with which sensitization of an antigen or the antibody was carried out, and the solution which a surfactant is mixed at least and changes are mixed according to this method, the erythrocyte in the blood which checks a reaction and measurement is first destroy d with the surfactant in a solution (the 1st reaction). (hemolysis) The leached moiety and antibody, or an antigen reacts after that with the insoluble support particle by which sensitization was carried out, and an antigen-antibody reaction is performed promptly (the 2nd reaction). That is, a direct antigen-antibody reaction reagent can be presented with a whole blood. By the method given in JP,9-274041,A, although hemolyze blood with a surfactant, it is made to react by having poured in the solution containing the insolubl support particle (latex liquid) by which sensitization of an antibody or the antigen was carried out at least to the component and reactions were two stages, since a reaction is performed in one stage, the time and effort of measurement can also be saved sharply.
[0008] In the measuring device of the antigen-antibody reaction matter which measures the predetermined antigen in blood, or the concentration of an antibody, without the measuring device of this invention separating a corpuscle from the extracted blood The solution which distributed the insoluble support particle by which sensitization of the antibody or antigen predetermined [aforementioned] to an antigen or an antibody was carried out to a reaction cell and this reaction cell, A pouring means to pour in th extracted blood and the solution which blood and a surfactant are mixed at least among the solutions which a surfactant is mixed at least and change, and changes, A mixed means to mix the liquid in the aforementioned reaction cell, and an optical irradiation means to irradiate light at the liquid in the aforementioned reaction cell, It is characterized by providing an amount detection means of transmitted lights to detect the amount of transmitted lights which penetrated the liquid among the light by this optical irradiation means, and a concentration fixed quantity means to ask for the aforementioned predetermined antigen in the aforementioned blood, or the concentration of an antibody based on the amount of transmitted lights which this amount detection means of transmitted lights detected.
[0009] In this equipment, if a pouring means pours in blood and the solution which a surfactant is mixed at least and changes, an operator will put in the solution which distributed the insoluble support particl by which sensitization of an antibody or the antigen was beforehand carried out to the reaction cell, and will set to a pouring means. Moreover, if the solution which the solution, the blood, and the surfactant with which the pouring means distributed the insoluble support particle to which sensitization of an antibody r th antigen was carried out are mixed, and changes is poured in, an operator will set an empty reaction cell to a pouring means. And a mixed means mixes the liquid in a reaction cell, and an optical irradiation means irradiates light at the liquid. A concentration fixed quantity means asks for the aforementioned predetermined antigen in the aforementioned blood, or the concentration of an antibody based on th amount of transmitted lights which the amount detection means of transmitted lights detected.
[0010] Moreover, let a surfactant be a nonionic surface active agent in the above-mentioned method and equipment. According to this, it can measure with a broad and sufficient precision from low concentration to high concentration. As a nonionic surface active agent, polyoxyethylene alkyl phenyl ether and polyoxyethylene alkyl ether are suitable.
[0011] Moreover, in the above-mentioned method and equipment, if the antigen of the measuring obj ct is C reactivity protein, it can be measured with a sufficient precision.
[0012] Moreover, in the above-mentioned method and equipment, if the wavelength of light to irradiat is the range which is 600-2000nm, a suitable measurement result will be obtained.
[00013]
[Embodiments of the Invention] Drawing 1 shows the reactor used for this measuring method. Explanation of this equipment sets up the light of the light source 1 so that a spectrum may be carried out with a spectroscope 2, among these the light of wavelength with very little absorption may be irradiated by the reaction cell 3 to hemoglobin etc. The reaction cell 3 is created by the transparent member, and th light which penetrated this reaction cell 3 is changed into an lectrical signal with a d tector 4. The output of this det ctor 4 is Log. It r sults in a converter 5, logarithmic transformation is carried out here, and, next, it is A/D. It results in a converter 6, and is changed into digital value here, and the value is displayed with a drop 7.
[0014] The gestalt of this operation explains how to measure the concentration in blood of a certain matter X which produces an antig n-antibody reaction. In the gestalt of this operation, the solution which

distributed the polystyren lat x particle as a solution which distributed the insoluble support particle to which sensitization of the antigen or antibody which produces an antigen-antibody reaction to Matter X was carried out is us d. Moreover, the hemolysis diluent which the buffer solution mixed with the surfactant is used as a solution which the surfactant mixed at least.

[0015] A tester first pours in and agitates the solution (latex liquid is called below) with which the antigen and the antibody sensitization polystyren latex particle were distributed by the reaction cell 3, the extracted blood, and a hemolysis diluent. Here, each liquid may be simultaneously injected into the reaction cell 3, and you may pour it in at a time 1 liquid. There is especially no turn of pouring. The hemoglobin which an erythrocyte hemolyzes here and is contained in the erythrocyte is eluted.

[0016] The antigen and antibody sensitization polystyrene latex particle (polystyrene latex particle which combined the antigen or the antibody) which were distributed to the rose rose in the solution at this time ar condensed by the antigen-antibody reaction, and the particle size on appearance increases. If an aggregate grows and sees with advance of an agglutination reaction and the upper particle size increases, the amount of transmitted lights will decrease. The grade of the particle-size increase by such insoluble support particle agglutination reaction is decided by concentration of the antigen contained in a sample, or an antibody. Therefore, it depends for the amount of transmitted lights on the concentration of the antigen contained in a sample, or an antibody. Although the polystyrene latex particle was used as an insoluble support particle here, you may use mineral-matter impalpable powders, such as organic polymeric-material impalpable powders, such as synthetic-resin powder, a bacterium, and a piece of a cell, and an impalpable powder of a metal, an inorganic oxide, a mineral, a silica, an alumina, and a silica alumina, for others. However, suppose substantially that it is insoluble to a liquid medium.

[0017] Next, a tester makes this reaction cell 3 penetrate the light from a spectroscope 2, reads the display of a drop 7 immediately, reads the display of a drop 7 in this time a in b at the time after predetermined-time t1 progress further, and records each value. The amounts Ita and Itb of transmitted lights at each time by this Opposite numeric values LogIta and LogItb It is obtained.

[0018] absorbance A since it is expressed with A=Log (Io/It) when the amount of incident lights is set to Io and the amount of transmitted lights is generally set to It -- the amount of transmitted lights -- Ita from -- Itb the time of changing -- change part deltaA of the absorbance deltaA=Log (Ita/Itb) =LogIta-LogItb can be calculated and calculated.

[0019] Next, a tester is deltaA for which it asked with reference to the calibration curve of the matter X in which the relation of the changed part and concentration of an absorbance is shown. It asks for corresponding concentration.

[0020] The concentration of Matter X differs here instead of the blood in which the calibration curv carried out [above-mentioned] extraction, respectively. n sorts of blood serums, i.e., n sorts of standard solutions, each concentration of whose is known It is a changed part deltaA1 of an absorbance lik the above. deltaA2, deltaA3 .... If deltaAn is calculated and this creates the regression line of the relation of th changed part and concentration of an absorbance, it can ask like drawing 2 .

[0021] In the above-mentioned example, it is asking for the concentration of Matter X directly with reference to the calibration curve from deltaA for which it asked by transmitted light measurement of a whole blood. However, this calibration curve is created based on the standard solution of a blood serum. And the concentration of the matter X in a whole blood (the erythrocyte is included) is thinner than the concentration of the matter X in a blood serum (the erythrocyte is not included). For this reason, if it asks for the concentration of Matter X directly with reference to the above-mentioned calibration curve from the above-mentioned deltaA, some error will arise with the concentration of the actual matter X. Then, this deltaA is amended, and it changes into the value corresponding to the concentration of the matter X in a blood serum, and asks for the concentration of Matter X with reference to the above-mentioned calibration curve using this value.

[0022] The following relational expression using the hematocrit value HCT which measured beforehand and was calculated performs this amendment.

(deltaA after amendment) =deltaAx {100/(100-HCT)} -- (1)

If it does in this way, accuracy can be asked more for th concentration of Matter X.

[0023] When asking for th CRP concentration in blood using the above-m ntioned method, the r agent and quantity from which a suitable result is obtained, and light wave length are described below.

[0024] The standard solution for reagent (1) calibration-curve cr ation; ERUPIA ac CRP reference

standard 0, 1.5, 3.5, 7.0, 14.0 mg/dl (the product made from YATORON, tradename)
(2) Latex liquid; anti-Homo sapiens CRP sensitization latex ERUPIA ace CRP-L (the product made from YATORON, tradename)
(3) The solution with which it com s to mix a surfactant (hemolysis diluent)
Buffer solution; phosphate buffer solution (you may add salts, such as NaCl and an ammonium oxalate, into the buffer solution)
Surfactant; the nonionic surface active agent is suitable.
(Example 1)
[Formula 3]
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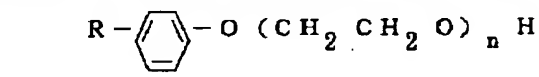

$R = C_m H_{2m+1}$ $(1 \leq m \leq 18)$ 、

n= 10 (10 - 100 grade) or more are suitable.
Especially, the polyoxyethylene dodecyl phenyl ether whose polyoxyethylene undecyl phenyl ether R whose polyoxyethylene desyl phenyl ether R whose polyoxyethylene nonylphenyl ether R whose R is the polyoxyethylene octyl phenyl etherC9H19 whose R isC8H17 is C10H21 is C11H23 is C12H25 is suitable.
(Example 2) Polyoxyethylene-alkyl-ether R-O-(CH2 CH2 O) n HR=Cm H2m+1 (1<=m<=18) ** is suitable.
By using these surfactants of non-ionicity, it is possible to measure broadly from low concentration to high concentration.
[0025] Quantity (rate of a volume ratio)
5-50 have the optimal hemolysis diluted solution (it consists of the buffer solution and a surfactant) to the whole-blood sample 1. As for the surfactant concentration in a hemolysis diluted solution, about eight are suitable for pH of about 1.0wt% and the buffer solution. In addition, according to the amount of the whole-blood sample used about the addition of latex liquid, it adds suitably.
[0026] Conversion becomes it bad that a hemolysis diluted solution is five or less to the whole-blood sample 1, and hemolysis stops working or more by 50. Moreover, a suitable result will be obtained if the above-mentioned operating wavelength is about 600-2000nm of near-infrared light.
[0027] The example of a time change of an absorbance in case samples differ in drawing 3 as reference here is shown. Reaction curves 1 and 3 are the reactions at the time of using plasma for a sample. That is, a reaction solution is plasma + hemolysis diluted solution + latex liquid. Reaction curves 2 and 4 are the reactions at the time of using a whole blood for a sample. That is, a reaction solution is whole-blood + hemolysis diluted solution + latex liquid. It is reaction progress by the method currently performed conventionally uses plasma, and, as for reaction curves 1 and 3, it turns out that the absorbance (ABS) is increasing with time. Reaction curves 2 and 4 are what used the whole blood, and it turns out that the absorbance is increasing with time. And if the reaction curves 1 and 3 by the conventional method are compared with the reaction curves 2 and 4 by the method of this invention, although conversion will fall from the former in the latter, it turns out that both approximate. However, since the concentration of CRP in a whole blood is thinner than CRP in plasma, Using the hematocrit value HCT measured independently, by the above-mentioned (1) formula and the same following formula, if it is an amendment Change part deltaAbs 1 and 3 of the absorbance when using the plasma of reaction curves 1 and 3, and reaction curv s 2 and 4 Change part deltaAbs 2 and 4 of the absorbance when using a whole blood serves as the almost same value.
(deltaAbs 2 and 4 after amendment) =(deltaAbs 2 and 4 when using whole blood) x {100/(100-HCT)}
here — absorbance of absorbance-0sec of deltaAbs2 and 4=300sec it is .
[0028] Although the above is the example of CRP measurement, if the antigen or antibody combined with the latex particle of latex liquid is replaced with, the rheumatoid factor in blood (RF), anti-streptolysin O (ASO), etc. can be measured similarly.
[0029] An optical irradiation m ans to irradiate light in the above-mentioned example at a reaction cell is Light Emitting Diode to the light source, although constituted from the light source and a spectroscope. The spectroscope is unnecessary if it uses. Moreover, although churning performed mixture of a solution, shake may perform.

[0030] Next, the equipment which measures the concentration in blood of the above-m ntioned matter X by such method is explained. The whole composition is shown in drawing 4 .
[0031] As for the light of the light source 8, the light of wavelength with very little absorption is generated to hemoglobin etc., and this light is set up so that the reaction cell 9 may irradiate. The reaction cell 9 is created by the transparent member, and th light which penetrated this reaction cell 9 is changed into an electrical signal with a detector 12. The output of this detector 12 is A/D. It results in a converter 13, and it is changed into digital value here and made to result in a microcomputer 14.
[0032] If blood and the reagent transfer pipet 10 hold blood, a hemolysis diluted solution, and the sensitization latex liquid that produces an antigen-antibody reaction to Matter X, respectively and has directions of a microcomputer 14, it will inject into the reaction cell 9 the liquid chosen according to the directions. Churning equipment 11 is equipment which agitates the liquid held in the reaction cell 9 according to directions of a microcomputer 14. A printer 15 prints the data outputted from the microcomputer 14. A microcomputer 14 is A/D while consisting of input units, such as an interface for delivering and receiving data with an operation, the central processing unit (Following CPU being called) which performs control, the main memory which consists of a ROM and RAM, and the exterior, and a keyboard, and controlling each part of this equipment. The data from a converter 13 are processed. The flow chart of operation of CPU is shown in drawing 5 .
[0033] Operation of this equipment is explained with reference to drawing 5 .
[0034] It is directed that CPU injects blood, a hemolysis diluted solution, and latex liquid into the reaction cell 9 to blood and the reagent transfer pipet 10 (Step 101). Thereby, blood and the reagent transfer pipet 10 inj ct blood, a hemolysis diluted solution, and latex liquid into the reaction cell 9. Next, CPU directs churning to churning equipment 11 (Step 102). Thereby, churning equipment 11 agitates the liquid of the reaction cell 9 predetermined-time t2.
[0035] Next, make the light source 8 emit light immediately after this churning, the light source 8 is made to emit light after a predetermined time t3 from the time further, and CPU is A/D at each time. It is based on data from a converter 13, and is deltaA a changed part of the difference of the absorbance at both the times, i.e., an absorbance. It asks (Step 103).
[0036] Next, CPU is deltaA for which it asked at Step 103 with reference to the changed correspondence tabl of the blood drug concentration of Matter X, and an absorbance which it was beforehand inputted and has b n memorized to main memory. It asks for the blood drug concentration of the corresponding matter X (Step 104).
[0037] Next, CPU is deltaA a changed part of the absorbance for which it asked at the data of concentration for which it asked at Step 104, and Step 103. Data are outputted to a printer 15 (Step 105). A print r 15 prints these data.
[0038] The above-mentioned correspondence table is a changed part deltaA1 of the absorbance about two or more sorts of each blood serum liquid each concentration of whose the concentration of Matter X differs instead of the above-mentioned sample, and is known using this equipment beforehand. deltaA2, If it asks for deltaA3 —, since the changed regression line of a concentration-absorbance will be called for by this, it can create from this straight line.
[0039] with this equipment, it asks for deltaA by transmitted light measurement of a whole blood (Step 103), and asks for the concentration of Matter X directly with reference to a calibration curve from this deltaA — **** (Step 104) — after asking for deltaA at Step 108, the above-mentioned (1) formula amends this deltaA, and you may make it ask for the concentration of Matter X with reference to a calibration curve from deltaA after this amendment The hematocrit HCT used for this amendment is calculated by anoth r measurement, and the main memory of a microcomputer 14 is made to memorize it beforehand. If it do s in this way, accuracy can be asked more for the concentration of Matter X.
[0040] the light source an optical irradiation means emits the light of many wavelength, such as a tungsten lamp, also in this equipment, and the thing by which spectroscopes are consisted of — you may be — only — Light Emitting Diode you may be . Moreover, although the mixed means was used as churning equipment, it is replaced with this and is good also as a shak r. Moreover, although it was made for blood and a reagent transfer pipet (pouring means) to also pour in latex liquid, it is made the composition which can pour in only blood and a hemolysis diluent, and you may make it set to this blood and reagent transfer pipet that to which the operator put latex liquid into the reaction cell beforehand with this equipment.
[0041]

[Effect of the Invention] Sinc according to the method of this invention, and equipment a whole blood can be poured into a direct antigen-antibody reaction reagent in case the concentration of the antigen-antibody reaction matt r in blood is m asured, pretreatment which carries out centrifugal separation of the bl od becomes unnecessary, and measurement is possible using the same sample as the object for constituent-of-blood measur ment. Mor over, lik a method given in JP,9-274041,A, a whole blood is hemolyzed with a surfactant and operation of pouring it into an antigen-antibody reaction reagent also becom s unnecessary. Therefore, with the method of this invention, and equipment, an institution without a centrifugal separation machine can also be measured and measurement with simply still quicker operation which the time and effort of pretreatment does not require can be performed.

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DESCRIPTION OF DRAWINGS

[Bri f Description of the Drawings]
[Drawing 1] Drawing showing the composition of the reactor used for this invention method.
[Drawing 2] Explanatory drawing of calibration-curve creation used for this invention method.
[Drawing 3] Drawing showing a time change of the absorbance of various liquids.
[Drawing 4] Drawing showing the composition of this invention equipment.
[Drawing 5] Drawing for explaining operation of the equipment shown in drawing 4 .
[Description of Notations]
1 Eight Light source
3 Nine Reaction cell
4 12 Detector
7 Drop
10 Blood and Reagent Transfer Pipet
14 Microcomputer

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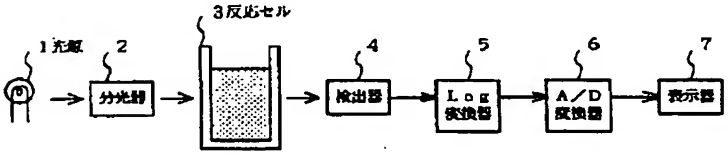
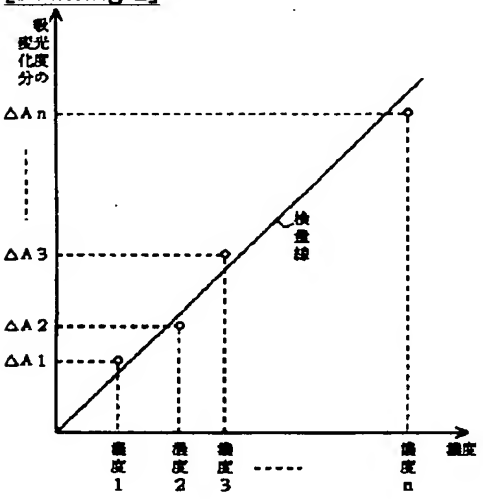
DRAWINGS

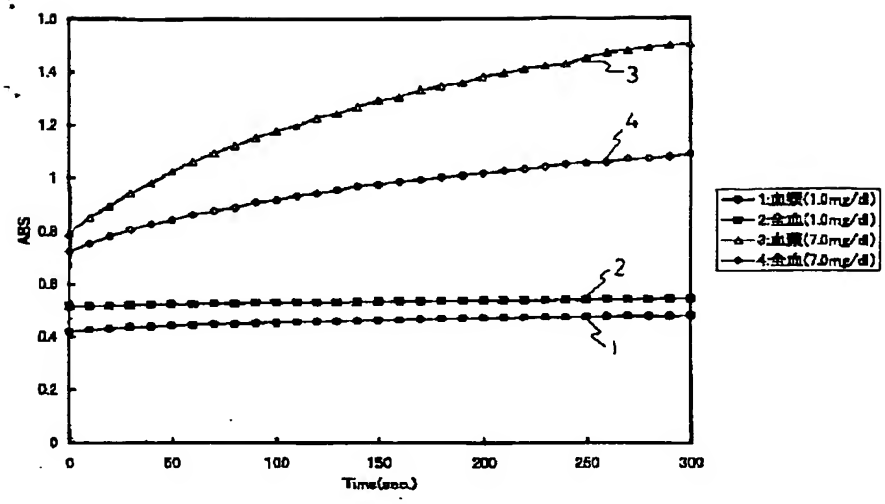
[Drawing 1]



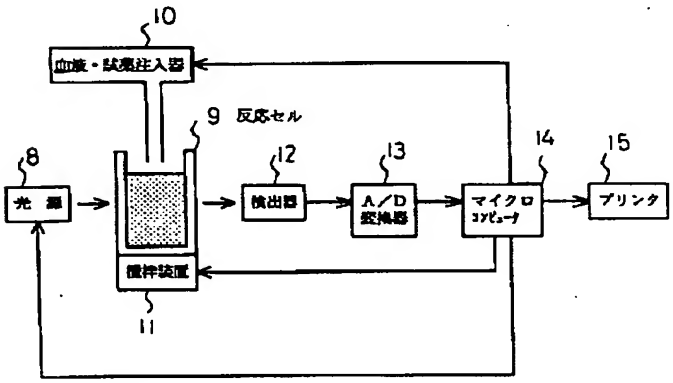
[Drawing 2]



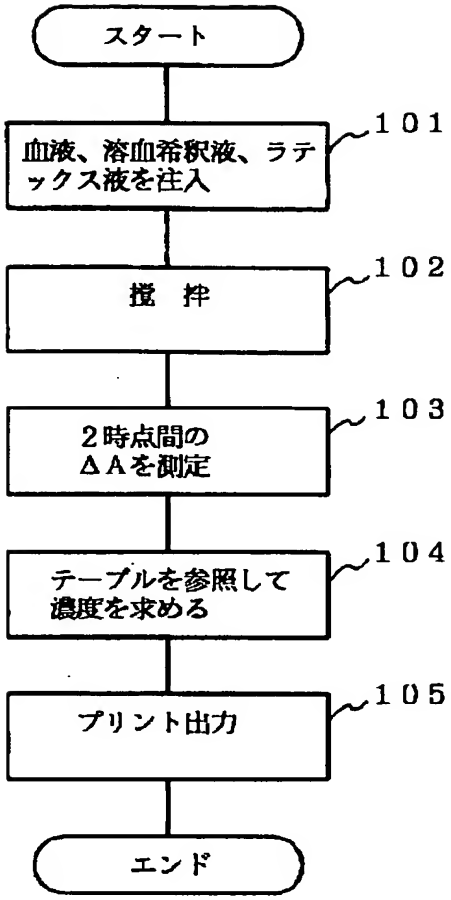
[Drawing 3]



[Drawing 4]



[Drawing 5]



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